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Brain Endothelial Cell Death: Modes, Signaling Pathways, and Relevance to Neural Development, Homeostasis, and Disease

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Abstract Emerging evidence indicates that brain microvascular endothelial cells play a critical role in brain development, maturation, and homeostasis. Acute or chronic insults, including oxidative stress, oxygen–glucose deprivation, trauma, infections, inflammatory cytokines, DNA damaging agents, β-amyloid deposition, and endoplasmic reticulum stress induce brain endothelial cell dysfunction and damage, which can result in cell death. The homeostatic balance between endothelial cell survival and endothelial cell death is critical for brain development, remodeling, and repair. On the other hand, dysregulation of brain endothelial cell death exacerbates, or even initiates, several inflammatory, ischemic, and degenerative disorders of the central nervous system. In here, the morphological, biochemical, and functional characteristics of the brain endothelium and its contribution to brain homeostasis will be reviewed. Recent insights into modalities and regulatory pathways involved in brain endothelial cell death will be described. The effects of regulated and dysregulated endothelial cell death leading to angiogenesis will be outlined. The relevance of brain endothelial cell dysfunction and death to disease processes will be discussed with special reference to recent findings that could help translate current knowledge on brain endothelial cell apoptosis into new therapeutic strategies for the treatment of certain neurological disorders.

Keyword Brain · Endothelium · Apoptosis · Signaling pathways · Neurogenesis

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Introduction

Because of the extended life expectancy and aging of populations, the number of people affected by neurological disorders is predicted to increase worldwide [1, 2]. Diseases of the brain are not only associated with high mortality, morbidity, and long-term disability, but they also have significant psychosocial and economic consequences and are likely to become a threat to public health globally, as recently reported by the World Health Organization [3–5]. Therefore, there is a critical need to foster better preventive and therapeutic strategies to decrease the burden of neurological disorders.

Recent advances in neuronal research have contributed to the emerging concept that the structural and functional integrity of the central nervous system depends on the coordinated activity of the neurovascular unit [6] and that the cerebral microvasculature plays an essential role in orchestrating the interactions between neurons and the remaining neural environment [7]. Brain endothelial cells provide a dynamic interface between circulating blood and the brain parenchyma and are essential for brain homeostasis [7]. In addition to their participation in the regulation of the vasomotor tone, blood cell trafficking, modulation of platelet adhesion, and neovascularization, cerebral endothelial cells play a critical role in maintaining the integrity and

function of the blood–brain barrier (BBB) and in supporting neuroaxonal growth and brain plasticity [8–10].

Injuries to the endothelial cell lining, triggered by inflammation and other insults, can result in endothelial death which contributes to the pathogenesis of diverse neurological disorders [11]. Accordingly, results from experimental animal models and clinical studies suggest that several inflammatory, ischemic, and degenerative disorders of the central nervous system are exacerbated, if not initiated, by brain microvasculature injury and death [12–14]. Therefore, effective therapeutic strategies to protect neuronal functions, to preserve the integrity of the BBB, and to ensure brain repair should be targeted toward the brain microvasculature.

This review highlights the current information on the types of cell death responses that occur in brain endothelial cells and on the mediators involved in the regulation of these responses. The major signaling pathways involved in death responses of brain endothelial cells will be reviewed, together with the relevance of brain endothelial cell death to brain homeostasis and disease.

Morphological and Functional Characteristics of the Brain Endothelium

Brain endothelial cells possess unique morphological and functional features compared to endothelial cells derived from vessels of peripheral organs [8]. The cerebral endothelium is a continuous endothelium characterized by a lack of fenestration and by the presence of complex tight junctions between endothelial cells [15]. These features confer to the cerebral endothelium a resistance of 1,000–2000 Ωcm^2, which is 100 times higher than the resistance of peripheral endothelial cells [16]. Moreover, brain endothelial cells possess an increased number of mitochondria and a low number of pinocytotic vesicles that limit endocytosis and transcytosis [17, 18]. These anatomical characteristics allow brain endothelial cells not only to rapidly deliver oxygen, glucose, and other nutrients to meet the high metabolic demands of the brain but also to participate, together with astrocytes, pericytes, and perivascular microglia, to the formation of the BBB, a cellular structure whose function is to protect the brain from metabolic, environmental, infectious, and toxic insults in addition to supplying nutrients and contributing to the creation of a parenchymal microenvironment conducive for neuronal function and activity [14] (Fig. 1).

The importance of the cerebral endothelium in brain homeostasis and disease is also emphasized by the close anatomical and functional relationship of brain endothelial cells with the remaining neural parenchyma [19]. The brain endothelium and the neural cells, including neurons, astrocytes, pericytes, and glial cells, together with components of the extracellular matrix, form an anatomically and functionally integrated system network, termed the neurovascular unit [6] (Fig. 1). The paracrine interactions between endothelial cells and neurons within the neurovascular unit are critical for brain plasticity and cognitive functions [7, 20]. Signals emanating from neural cells regulate vascular tone and BBB function, thus ensuring the proper metabolic exchange between blood and the brain parenchyma [21, 22]. On the other hand, trophic factors secreted from endothelial cells provide nourishment and protection to neurons, while glial cells protect both neurons and the endothelium [23, 24]. Interactions between endothelial cells and the extracellular matrix are also crucial for the stability of the vascular wall [25]. Moreover, cues emanating from the extracellular matrix are essential for signaling for endothelial cell survival, migration, and differentiation [26]. Thus, the finely tuned communication between endothelial cells and the remaining components of the neurovascular unit ensures the anatomical and functional integrity of the brain.

In addition to its active contribution to the BBB anatomy and function and to the neurovascular unit, emerging evidence indicates that the cerebral endothelium plays a critical role in neurogenesis during development and in the postnatal brain [27, 28]. In the vascular niches of the brain, normal neural stem cells, which are located in the hippocampus and subventricular zone, are in close physical contact with brain endothelial cells, and this direct cell contact appears to be an essential prerequisite for the matrix-dependent neurotrophic effect of brain endothelial cells on neural stem cell self-renewal and differentiation [29, 30] (Fig. 1). In addition, in these vascular niches, endothelial cells supply soluble factors and mediators that in turn regulate neural stem cell functions [31–33]. Recent experimental studies suggest that brain endothelium-derived neurotrophic factors have also a protective effect on neurons and imply the requirement of a functionally active endothelium for anatomical and functional recovery of neuronal activity after trauma, ischemia, or oxygen deprivation [31–34].

Acute or chronic insults, including oxidative stress, oxygen–glucose deprivation, trauma, infections, pro-inflammatory cytokines, DNA-damaging agents, and deposition of amyloid-β peptide (Aβ) fragments [21, 35–39] impose major challenges to brain endothelial cells as these cells are somewhat protected by the presence of a skeletal structure and therefore evolutionarily more sensitive to external insults than endothelial cells from peripheral organs. These insults can lead to microvascular damage and negatively affect many aspects of endothelial cell functions, including survival. Decreased survival of brain endothelial cells in turn compromises the anatomical and

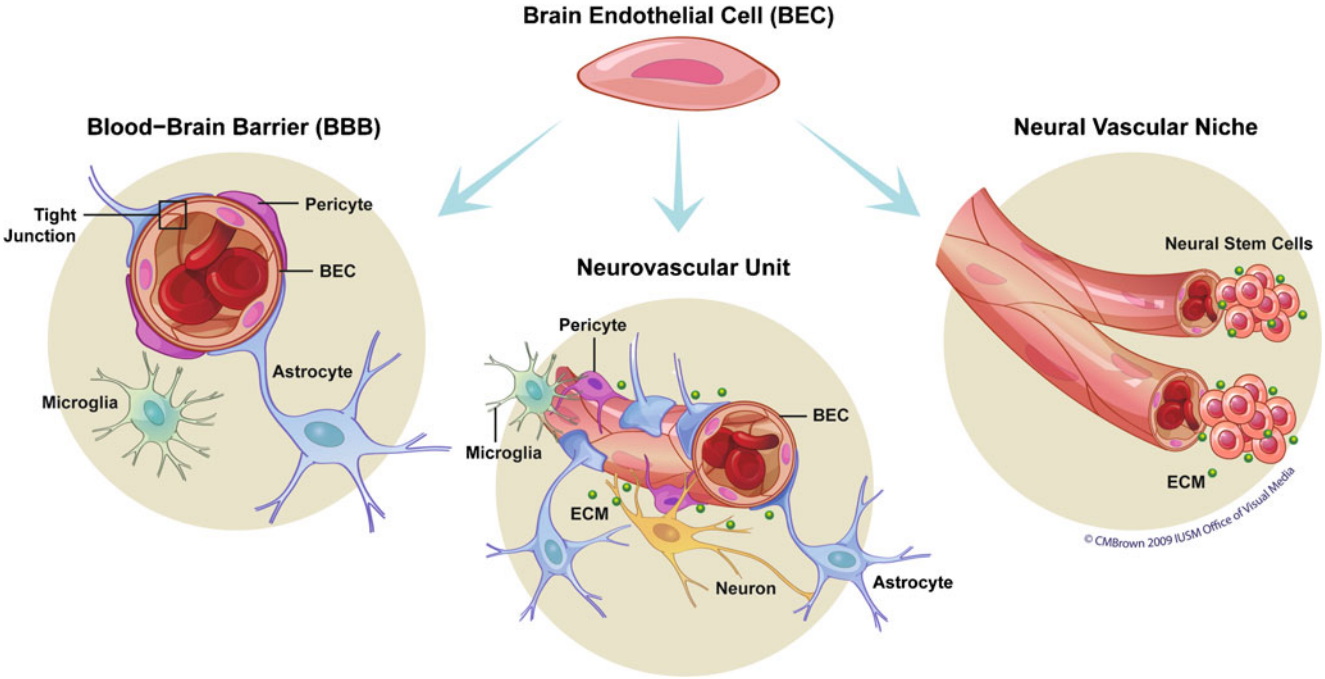

Fig. 1 Anatomical and functional interactions between brain endothelial cells and neural cells. Brain endothelial cells (*BECs*) interact with neural cells to form three distinct anatomical and functional structures that are essential for brain homeostasis: (1) the blood–brain barrier (*BBB*); (2) the neurovascular unit; and (3) the neural vascular niche

functional integrity of the neurovascular unit, the BBB, and the neural vascular niche, leading to the disruption of brain homeostasis and disease [13, 40, 41].

Modes, Agonists, and Mediators of Brain Endothelial Cell Death

During their life span, brain endothelial cells are subjected to a variety of noxious and potentially death-inducing stimuli that can lead to their demise (Table 1) [35–39]. In addition, the brain endothelium can be affected, as an innocent bystander, by injuries to the other cellular components of the neurovascular unit [20]. Moreover, disruption in the interactions of brain endothelial cells with the surrounding perivascular and neural cells or with the extracellular matrix can negatively affect their survival [30]. Depending on the type of death signals, their duration, and intensity, brain endothelial cells can undergo distinct modes of death, as discussed below.

Necrosis/necroptosis Necrosis is a form of cell death that occurs in the central nervous system, mostly as a consequence of ischemia or radiation therapy [42, 43]. Morphologically, necrosis is characterized by the loss of plasma membrane integrity, organelle swelling, mitochondrial dysfunction, and the lack of the typical apoptotic features of DNA cleavage [44]. Moreover, almost invariably, necrotic cell death triggers an inflammatory and immune response [44]. While necrosis was previously considered an accidental form of cell death, recent evidence indicates that like apoptosis, the necrotic response is coordinated by the activation of specific regulatory pathways [44]. This regulated form of necrosis, which is known as necroptosis, appears to have an important role in many disease processes of the central nervous system [45]. Consistent with this possibility, recent results from a genome-wide siRNA screening analysis revealed that a large subset of genes involved in the regulation of necroptosis are expressed in the immune system and in the brain [46]. Moreover, recent studies demonstrated the occurrence of necroptosis in the brain as a result of ischemia and excitotoxicity [47]. However, the contribution of necroptosis to brain endothelial cell death remains to be determined.

Apoptosis Apoptosis is a fundamental cellular response that plays a crucial role in development and homeostasis [48]. Genetic studies have demonstrated that deletion of pro-apoptotic genes results in abnormalities in the central nervous system [49]. In addition, elimination of dysfunctional cells is critical for tissue and organ homeostasis [50]. However, when inappropriately regulated, apoptosis can also promote, contribute to, and even exacerbate disease processes [51].

According to the recent recommendations from the Nomenclature Committee on Cell Death [44], apoptosis is

Table 1 Modes and mediators of brain endothelial cell death

Insults	Mediators	Modes	References
Inflammation	TNF, CD40-CD40L	Apoptosis extrinsic	[62–64]
	CD47, Thrombospondin-1	TNF-R1	[64]
	LPS	Apoptosis extrinsic	[98]
	FasL	Indirect	[66, 67]
Infections	*Streptococcus pneumoniae*	Apoptosis caspase-dependent and independent	[39, 68, 88]
	Plasmodium falciparum	Apoptosis extrinsic and intrinsic	[70, 72]
	Escherichia coli	ER-dependent	[90]
	HIV	Apoptosis caspase-dependen	[69]
	African Trypanosomiasis	Apoptosis intrinsic	[71]
Irradiations	Ceramide	Necrosis	[43, 82, 83]
Ischemia	Glutamate, NO, ROS	Apoptosis caspase-dependent, necrosis	[35, 77, 80]
	Ca++, lipids	Necrosis, necroptosis, calpain	[73, 78, 81]
Excitoxicity	Glutamate	Necroptosis	[47]
Degenerative diseases	β-amyloid, ROS, NO, Ca++	Apoptosis intrinsic and extrinsic	[73–76]
		ER stress	[74]
Toxic agents	Cadmium, pesticides	Apoptosis intrinsic	[84, 85]
Cell detachment	Integrins, ceramide	Anoikis	[44, 86, 87]

associated with distinct morphological features, including rounding up of cells, cellular and/or nuclear picnosis, chromatin condensation, and DNA fragmentation. In the initial phase of apoptosis, the integrity of the plasma membrane and cytosolic structures is conserved [44]. However, as the apoptosis response progresses, disruption of the plasma membrane can occur, especially if apoptosis is accompanied or followed by necrosis [44].

The apoptotic mode of cell death is a complex response, orchestrated by, but not necessarily dependent upon, the caspase family of cysteine proteases, a group of cytosolic enzymes present in healthy cells as inactive proenzymes [52]. Upon activation, caspases cleave target substrates at specific aspartic acid residues [52]. According to their role and activation kinetics, caspases are classified as apical caspases, effector caspases, and inflammatory caspases. The apical caspases, which comprise caspase-2, caspase-4, caspase-8, caspase-9, caspase-10, and caspase-11, are activated in the initial phase of the apoptotic cascade and are responsible for linking death signals to the effector caspases [53]. The effector caspases, which include caspase-3, caspase-7, and caspase-6, along with other enzymes such as calpain and cathepsins, are directly responsible for the execution of cell death, which they execute by cleaving specific target substrates [53–56]. In contrast, the inflammatory caspases, which include caspase-1, caspase-5, and caspase-12, act to link cytokine signaling to inflammatory responses which may or may not, depending on cell type and microenvironmental factors, lead to cell death signaling [57].

Two distinct pathways are involved in the regulation of caspase activation. The extrinsic pathway, or death receptor-mediated pathway, is triggered by ligation of members of the TNF superfamily of cytokines, including TNF-α, Fas ligand (FasL), and TNF-α-related apoptosis inducing ligand to the death receptors [58]. This event is followed by recruitment of the cytosolic adapter protein FADD within the death-inducing signaling complex and caspase-8 activation [58]. The intrinsic pathway is initiated by mitochondrial release of cytochrome c which, together with Apaf-1 and deoxyATP, oligomerizes to form the apoptosome complex, resulting in the activation of caspase-9 [59]. Mitochondrial perturbations can induce the release of second mitochondrial-derived activators of caspases (Smacs), which in turn promote apoptosis by binding and inactivating members of the inhibitor of apoptosis protein (IAP) family [60]. Caspase activation, especially in mitochondrial-dependent signaling, is often orchestrated by the bcl-2 family, a large group of proteins that can mediate pro-apoptotic or anti-apoptotic responses [61]. Thus, while Bcl-2 protects from apoptosis, other Bcl-2 family members, such as Bax, promote apoptosis [61].

Although the extrinsic and intrinsic pathways of apoptosis are activated by distinct death signals and are linked to activation of distinct caspases, there is ample evidence in support of a cross talk between both pathways. This cross talk may constitute an amplification loop that serves to potentiate the initiation and completion of the death program.

As shown in Table 1, brain endothelial cells are the target of a wide array of pro-apoptogenic stimuli. With regard to the extrinsic pathway of apoptosis, brain endothelial

cells express the whole repertoire of death receptors [62]. Pro-inflammatory cytokines, including TNF-α, CD40/CD40 ligand, CD47, and its ligand thrombospondin-1 induce apoptosis of brain endothelial cells by activating the extrinsic pathway [63, 64]. In contrast, brain endothelial cells are resistant to Fas ligand-induced cell death, although they express Fas receptor [65]. Fas ligand, however, induces disseminated endothelial cell apoptosis in a murine model of acute tissue damage produced by systemic transfer of allogeneic lymphocytes into immunodeficient mice [66]. This suggests that resistance of endothelial cells to Fas ligand-induced apoptosis results from the immunomodulator role of Fas ligand in the central nervous system [67].

Infectious agents, including bacteria, viruses, and parasites, can induce brain endothelial cell apoptosis via several mechanisms [39, 68–71]. For examples, *Streptococcus pneumoniae*, the main pathogen responsible for community-acquired bacterial meningitis in children and adults, triggers apoptosis via two distinct mitochondrial pathways [68]: a caspase-3-dependent pathway, which is activated by the physical interaction of the bacteria with the vascular endothelium of the BBB, and a caspase-3-independent pathway, which is initiated by the release of toxins, such H_2O_2 and pneumolysin, or by pro-inflammatory components of the bacterial cell wall [68]. Brain endothelial cell apoptosis is also detected during HIV infection [69]. The cytotoxic effect of HIV is mediated by the Tat envelop viral protein which induces caspase-dependent cell death [69]. Moreover, recent evidence suggests an important role of cerebral endothelial cell apoptosis in the pathogenesis of the neurological complications of malaria [70]. Using cultured human brain endothelial cells, Wassmer et al. demonstrated that *Plasmodium falciforme* induces brain endothelial cell apoptosis via TNF-α-dependent release of TGF-β [70]. In a murine model of cerebral malaria, $CD8^+$ T cell activation and production of perforin mediated caspase-3-dependent apoptosis of brain endothelial cells [72].

Apoptosis of brain endothelial cells is also triggered by deposition of amyloid-β peptide (Aβ) fragments in the vascular wall [73–75]. Aβ-induced brain endothelial cell death occurs via the extrinsic pathway of apoptosis in a caspase-8-dependent manner [76]. Deposition of Aβ also disrupts the mitochondria electron transport chain, resulting in increased production of reactive oxygen species, inhibition of ATP production, and subsequent apoptosis via the intrinsic pathway [76].

Ischemia-induced injury has recently emerged as a cause of apoptosis not only in neurons but also in the cerebral endothelium [77]. Importantly, brain endothelial cells appear to be more susceptible to ischemia-induced injury than glial cells or peripheral endothelial cells [78]. The damage inflicted by the ischemic insult to the cerebral endothelium is dependent on a variety of contributing factors, including reactive oxygen species, excitatory neurotransmitters, calcium perturbations, and products of lipid peroxidation, bioactive lipids, and inflammatory cells [78–81]. These factors can mediate distinct apoptotic responses. Thus, in a model of hypoxia-induced brain endothelial cell injury, Cheng et al. [79] demonstrated that apoptosis of brain endothelial cells was mediated by increased expression of p53, increased Bax-Bcl-2 ratio, and activation of caspase-3. Other studies have demonstrated nitric oxide-dependent activation of caspase-1, caspase-8, and caspase-3 in glutamate-induced apoptosis of brain endothelial cells [80]. Activation of the intrinsic mitochondrial pathway involving increase of Bax/Bcl-2 ratio, loss of mitochondrial membrane potential, and deoxyATP depletion has been implicated in oxidative stress-induced retinal and neural endothelial cell death [81]. In these studies, Quintou et al. [81] demonstrated that this pathway was activated by oxidative stress-induced production of thromboxane A_2 (TXA_2) which in turn promoted apoptosis via a calpain-dependent pathway.

The effects of radiation therapy on brain endothelial cell apoptosis have not been extensively studied, although radiation-induced toxicity is characterized by necrosis and apoptosis not only of neurons but also of the vascular endothelium. The release of pro-inflammatory cytokines, changes in the extracellular matrix environment, and generation of ceramide are likely to be major contributors of brain endothelial cell apoptosis following radiation therapy [82, 83]. Toxic agents, including pesticides and heavy metals, are also implicated in caspase-3-dependent apoptotic death of brain endothelial cells [84, 85].

Anoikis Adhesion of endothelial cells to the extracellular matrix is crucial for their survival and functions [86]. Detachment of endothelial cells from the extracellular matrix promotes an apoptotic response called anoikis, which refers to apoptosis induced by the loss of the attachment to the substrate [44]. Anoikis [87] has been reported in brain endothelial cells and is mediated by the production of ceramide.

Caspase-Independent Apoptotic Cell Death Recent evidence suggests a new mode of apoptotic cell death that involves a mitochondrial pathway initiated by the apoptosis-inducing factor (AIF) independent of caspase-9 activation [88]. AIF is released from the mitochondrial intermembrane and causes chromatin condensation and DNA fragmentation into large fragments. Interestingly, this mode of apoptosis occurs together with a caspase-dependent pathway in brain endothelial cells during *Streptococcus pneumoniae*-induced meningitis [68].

Endoplasmic Reticulum Stress and Apoptosis The endoplasmic reticulum (ER) plays a role in many apoptotic

pathways by either initiating cell death or by sensitizing the mitochondria to a variety of extrinsic and intrinsic death stimuli [89]. Under normal conditions, the main function of the ER is to process the proper folding and post-translation modification of proteins in addition to functioning as a reserve of intracellular calcium [89]. In response to cellular stress, unfolded proteins accumulate into the ER and are removed via the activation of the UPR pathway [89]. Thus, perturbations in ER homeostasis can lead to alteration of ER protein folding and apoptosis. Several pro-apoptotic proteins are located in the ER, including members of the Bcl family such as Bim [89]. In brain endothelial cells, ER-induced apoptosis was reported to occur after exposure to *Shiga* toxin from *Escherichia coli* [90]. The expression of the ER stress-related gene C/EBP homologous protein (CHOP/GADD153, growth arrest, and DNA damage-inducible protein) together with PERK increased significantly in human brain endothelial cells exposed to *Shiga* toxin 2 [90].

Signaling Pathways Involved in Regulation of Brain Endothelial Cell Apoptosis

The ability of cells to respond to changes in their environment is governed by a wide array of intracellular signaling pathways. These signaling pathways, rather than following a linear path, are temporally and spatially organized by signaling molecules, scaffolding proteins, and multi-domain adaptor proteins into highly interconnected networks that regulate a cell's decision to survive or die. The mitogen-activated protein kinase (MAPK) is an evolutionary conserved family of signaling components that plays a crucial role in integrating signals for the control of fundamental cellular processes such as cell proliferation, survival, differentiation, metabolism, motility, and apoptosis [91].

The majority of information pertaining to the signaling pathways involved in the regulation of apoptosis in vascular endothelial cells derives from studies performed in endothelial cells from peripheral organs. However, this information cannot be applied readily to the cerebral microvasculature because of the peculiar characteristics of brain endothelial cells. However, a few studies imply a role for MAPK kinase family members, including the extracellular signal regulated kinase (ERK), the c-jun amino terminal kinase (JNK), and p38-MAPK in the regulation of apoptotic responses in brain endothelial cells. Activation of ERK represents a canonical pathway for the regulation of cell growth and survival [91]. However, recent evidence suggests that in brain endothelial cells, activation of ERK, under certain environmental conditions, regulates apoptosis. Thus, recent work of Natasimba et al. [92] demonstrated that inhibition of ERK protects brain endothelial cells from apoptosis induced by oxygen–glucose deprivation. Moreover, ERK has been reported to partially mediate apoptosis of brain endothelial cells induced by exposure to pesticides [85].

JNK and p38-MAPK are activated in response to environmental and genotoxic stress and are involved in the regulation of apoptotic cell responses [93, 94]. The mechanisms by which JNK and p38-MAPK regulate apoptosis are complex, but studies performed in fibroblasts and transformed cells indicate that the pro-apoptotic effects of these kinases involve modulation of death receptors, phosphorylation of the bcl-2 family members, phosphorylation of 14-3-3 proteins, and upregulation of pro-apoptotic genes via the activation of transcription factors, including c-jun/AP1 and p53 [95–97]. Evidence for a role of JNK in the regulation of apoptotic pathways in brain endothelial cells derives from in vitro and in vivo studies. JNK mediates apoptosis triggered by exposure of cultured brain endothelial cell to lipopolysaccharide [98]. Moreover, in an animal model of cerebral vasospasm, inhibition of JNK ameliorated apoptosis of brain endothelial cells [99]. Recent studies also demonstrated that exposure of brain endothelial cells to Aβ activated a signaling cascade leading to the activation of Jun/AP1, Bim phosphorylation, release of Smac, and binding to IAP, suggesting the upstream involvement of JNK [100]. Activation of p38-MAPK has also been implicated in apoptosis of brain endothelial cells. Specifically, exposure of brain endothelial cells to cadmium or to ischemia–reperfusion induces apoptosis via a p38-MAPK-dependent pathway [84, 101]. The involvement of ERK, JNK, and p38-MAPK in apoptosis of brain endothelial cells, however, is cell context-specific, and under different experimental settings, these kinases may mediate cellular responses in brain endothelial cells other than apoptosis. Thus, we recently demonstrated that activation of JNK and ERK is involved in hepatocyte growth factor-stimulated migration of human brain endothelial cells [102].

Recent studies have highlighted a role for apoptosis-regulated kinase (ASK-1) in the signaling pathways of brain endothelial cell apoptosis. ASK-1 is a serine–threonine protein kinase that belongs to the MAPK kinase kinase family and is activated upstream to JNK and p38-MAPK by stimuli that increase the intracellular concentrations of reactive oxygen species [103]. Hsu et al. [104] elegantly demonstrated that Aβ induces apoptosis of brain endothelial cells via a signaling cascade involving protein phosphatase 2A (PP2A)-dependent activation of ASK-1, which in turn results in the activation of p38-MAPK, followed by phosphorylation of p53, activation of Bax, and apoptosis.

With regard to the involvement of bioactive lipid mediators in regulation of pathways leading to cerebral endothelial cell apoptosis, there is evidence to substantiate

an important role of ceramide released from neutral sphingomyelinase (nSMase) activation in response to irradiation and unligated integrins [83, 87]. Moreover, studies by Yin et al. [105] have shown that release of ceramide and activation of PP2A by Aβ leads to apoptosis via a pathway involving FOXO3a-dependent activation of Bim. Importantly, Bim expression was found to be activated in vessels isolated from a murine model of cerebral amyloid angiopathy [105]. There is evidence that other bioactive lipid mediators, including TXA_2 and 8-iso-prostaglandin $F_{2\alpha}$, mediate upstream events linked to apoptosis and necrosis of brain endothelial cells [78, 81], but the involvement of the MAPK family in these events has not been established.

Role of Brain Endothelial Cell Death in Angiogenesis

Angiogenesis, the process of new blood vessel formation from preexisting capillaries, constitutes the main mechanism involved in the formation of the brain vasculature during embryonic development [106]. In the postnatal brain where growth and development is complete, angiogenesis ceases and brain endothelial cells become quiescent, as evidenced by the fact that only 0.3% of the total population of endothelial cells in the adult rat brain incorporates ^{3}H-thymidine [107]. However, in response to environmental signals, the angiogenic response may be reactivated, stimulating endothelial cells to engage in a series of events initiated by degradation of the extracellular matrix and followed by proliferation, migration, and differentiation into capillary-like structures [108]. Following the integration of mural cells into the developing vessel wall, these immature vascular structures acquire the phenotype of mature blood vessels [109]. The ability of endothelial cells to appropriately mount an angiogenic response is essential not only for brain development and maturation but also for brain plasticity and repair. Elegant studies have highlighted the importance of angiogenesis for neurogenesis and neuroblast migration [110–112], suggesting that defective angiogenesis after ischemia, traumatic brain injuries, or degenerative insults can delay brain repair or even exacerbate the age-associated decline of cognitive functions.

Pro-apoptotic signals emanating from negative regulators of angiogenesis, such as angiopoietin-2 and thrombospondin-1 or integrin antagonists, contribute, together with positive regulators of angiogenesis, to modulate the angiogenic response occurring under physiological conditions [26, 113, 114]. Thus, apoptogenic pathways, when balanced with survival signals, serve to maintain the normal vasculature in a quiescent state. Moreover, apoptosis contributes to the formation of developing capillary structures by eliminating dysfunctional endothelial cells and by regulating the process of lumen formation [115]. Additionally, apoptotic endothelial cells stimulate sprouting of non-proliferating endothelial cells [116]. However, unbalanced apoptosis leads to inhibition of angiogenesis. Increased production of negative regulators of apoptosis or decreased production of positive regulators of apoptosis can occur as a result of several conditions [117–119]. For example, in the cold injury model, increased production of angiopoetin-2 occurs early after injury and is associated with increased expression of caspase-3, induction of apoptosis, and breakdown of the BBB [118]. Moreover, release of endostatin, a fragment of collagen XVIII that inhibits endothelial cell responses, has been detected from neurons of patients with Alzheimer's disease, suggesting a link between apoptosis, angiogenesis, and neurodegeneration [120].

Brain Endothelial Cell Death in Neurological Diseases

As shown in Table 2, several diseases of the central nervous system can be promoted or exacerbated by damage and death of brain microvascular endothelial cells. The contribution of brain endothelial cell apoptosis to neuronal damage is of particular interest in stroke because despite currently available therapies, stroke remains the third leading cause of death in the USA [2]. Recent studies demonstrated that the apoptotic process occurring in the penumbra, which refers to the area of viable tissue surrounding the ischemic core that if not treated can progress to irreversible infarction, targets neurons and endothelial cells [121]. Endothelial cell apoptosis worsens neuronal damage, as shown by the results of an experimental in vivo model of focal cerebral ischemia in which a correlation between the loss of vascular integrity and the degree of neuronal damage was observed [122]. Thus, apoptosis of brain endothelial cells in the penumbra has detrimental consequences on the functional integrity of the neurovascular unit as it disrupts BBB integrity, further reducing blood flow and exacerbating hypoxia-induced neuronal cell death [6, 23, 111, 112]. In addition, because of the critical role of angiogenesis in neurogenesis and synaptogenesis, apoptosis of brain endothelial cells negatively affects brain repair mechanisms which are essential for neurological recovery after stroke [111]. Several neuro-

Table 2 Involvement of brain endothelial cell death in the pathogenesis of neurological disorders

Diseases	References
Stroke	[121–123]
Subarachnoid hemorrhage	[124–126, 129]
Cerebral cavernous malformations	[130, 131]
Alzheimer's disease	[40, 120, 133]

protective therapies for stroke have shown promising results in experimental models and in preclinical trials [123]. However, these therapies have failed in clinical trails, possibly because they only target the neuronal population [123]. Therefore, anti-apoptotic strategies that target the cerebral microvasculature, in combination with standard therapies, hold the potential of ameliorating brain repair and neuroprotection in patients with stroke.

Apoptotic features involving the cerebral endothelium have been detected in experimental animal models and in patients with subarachnoid hemorrhage (SAH) [124, 125]. Despite therapeutic interventions, about 40–60% of patients with SAH die within the first 48 h of cerebral edema and vasospasm [126]. A main contributing factor to the development of brain edema and the occurrence of cerebral vasospasm following SAH is apoptosis of brain endothelial cells which is triggered by several factors, including pro-inflammatory cytokines and products of red cell catabolism [38, 127]. Endothelial cell apoptosis causes breakdown of the BBB, leading to vasogenic edema. Moreover, detachment of brain endothelial cells exposes the underlying vessel wall to vasoconstrictive and thrombogenic factors [128]. This, together with decreased endothelial production of vasodilators such as nitric oxide or prostacyclin, causes further vasoconstriction, which worsens the ischemic injuries induced by cerebral vasospasm [128]. Using a rat model of SAH, Park et al. [129] demonstrated that systemic treatment with caspase-3 inhibitor decreased apoptosis of brain endothelial cells, attenuated BBB permeability, reduced brain edema, and improved neurological score.

Dysregulation of apoptosis affecting the cerebral endothelium appears to play a role in the pathogenesis of cerebral cavernous malformations (CCMs). These lesions, which can occur in a sporadic or familiar manner, have debilitating clinical consequences as the majority of affected patients experience seizures, focal neurologic deficits, headaches, and cerebral hemorrhage [130]. Structurally, CCMs consist of a cluster of enlarged capillaries formed by a layer of endothelial cells with no astrocytes and few pericytes [130]. Recent studies demonstrated that patients with the CCM3 variant carry mutations in the programmed cell death gene PDCD10 [131]. Moreover, Chen et al. [132] demonstrated that overexpression of PDCD10 in cultured brain endothelial cell lines induced loss of integrin-mediated cell adhesion and apoptosis via activation of caspase-3 and p38MAPK. These results suggest that brain endothelial cell apoptosis plays a key role in the pathogenesis of CCMs.

Damage to the cerebral vasculature occurs also in neurodegenerative disorders such as Alzheimer's disease. The first indication in support of the involvement of endothelial cell dysfunction in the pathogenesis of Alzheimer's disease was provided in 1989 by the studies of Scheibel et al. [133]. Deposition of oligomeric Aβ fragments in the wall of the cerebral microvasculature (amyloid angiopathy) causes damage and apoptosis of brain endothelial cells via several mechanisms [73–76]. Damage to the endothelium and subsequent hypoperfusion and tissue ischemia is ultimately responsible for neuronal loss [40]. Importantly, the damage to the cerebral microvasculature constitutes an early event that precedes the neurodegenerative changes that ultimately lead to progressive loss of synapses and neurons. Additionally, recent studies demonstrated that patients with Alzheimer's disease have increased neuronal production of endostatin, an endogenous inhibitor of angiogenesis, suggesting a role for defective cerebral angiogenesis in the pathogenesis of hypoperfusion in patients with Alzheimer's disease [120]. Thus, defective angiogenesis could further exacerbate the neuronal damage inflicted by Alzheimer's disease. Given that cerebral endothelial dysfunction constitutes an early event in the pathogenesis of Alzheimer's disease, targeting the cerebral microvasculature could represent a rationale approach for the development of preventive strategies for patients at risk of developing Alzheimer's disease.

Conclusions

Brain endothelial cell death is a complex process involving distinct agonists and intracellular signaling pathways. Recent research has highlighted the important role that the cerebral endothelium plays in brain development, maturation, homeostasis, and disease processes. However, despite our increasing knowledge on the contribution of brain endothelial cell functions and dysfunction to brain pathophysiology, the molecular and signaling mechanisms that mediate cell death responses to a myriad of extracellular and intracellular stimuli are not well defined, and many questions remain unanswered. For example, how the continuously changing environment to which cells are exposed influences modalities of cell death, as well as regulatory and integrative apoptotic pathways, is not known. Knowledge on whether and how the interactions or lack of interactions between the endothelial cells and the other cells of the neurovascular unit dictate survival and apoptotic outcomes in response to environmental changes is crucial. More research is also needed to establish the relevance of apoptotic pathways in model systems that allow analysis of the potential pathways in disease models. Future research in these areas will hopefully increase our understanding on the mechanisms controlling life and death of the cerebral endothelium and provide the opportunity to foster future therapeutic strategies to achieve neuroprotection, enhance brain repair, and overall decrease the burden associated with cerebrovascular and neurodegenerative

disorders. These apoptotic signaling targets could also be exploited for the design of new anti-angiogenic therapies for brain tumors or to improve drug delivery through the BBB.

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